SOP 6: Estimating Density and Biomass of Red Imported Fire Ants

Use these procedures to estimate density and biomass of red imported fire ants (RIFA). The methods entail detecting and counting RIFA mounds and estimating mound size, which provides an estimate of ant biomass, and should be conducted only at sites identified as containing RIFA during the RAD (SOP 1).

Equipment and Supplies

- GPS
- Dowel rod (3/8 x 36 inch)
- Metric measuring tape (8 m)
- Data sheets (8 per site, plus extra), clipboard, pencil
- Camera

Safety Considerations

Pay attention to where you are walking or standing relative to RIFA mounds, check your shoes and pants frequently for RIFA. In densely vegetated areas where mounds are difficult to see, you may notice mounds by stepping on a "soft" spot, so make sure to step away from that location before checking for a mound. After the mound is disturbed, ants will crawl on vegetation, as well as the dowel rod and measuring tape, so be careful when measuring mounds.

Field Methods

The following steps should be done once per year from mid-March to May (earlier in southern Texas and later in northern Texas and Oklahoma):

- 1. For grassland plots, navigate to the NW corner of the plot (see SOP 1 and SOP 2)
- 2. Survey every other plant transect for RIFA (numbers 1, 3, 5, 7, 9, 11, 13, 15; see SOP 1; use a separate datasheet for each transect)
- 3. Search a 10 m wide belt centered along each transect (i.e., each belt transect extends 5 m to each side of a transect). Only mounds observed from the transect line and with part of the mound within 5 m of the transect line should be included
- 4. Measure the perpendicular distance (± 1 cm) from the transect to the center of each mound
- 5. Determine if each mound is active using the minimal disturbance method (disturb the mound by tapping with the dowel rod and then scraping/poking at the center of the mound with the dowel rod; a mound is considered active if ≥ 25 worker ants rapidly emerge from the mound following the disturbance; if ants emerge, but there are fewer than 25 individuals (i.e., the mound is not considered active), record a note about RIFA presence in the notes section of the data sheet)
- 6. Record the size of each active mound (length, width, height; ± 1 cm); length is measured as the longest axis of the mound, width as the shortest axis, and height as the highest point aboveground level; if more than 50 mounds are encountered per transect, then only record size data for the first 50 encountered mounds on each transect. Record the perpendicular distance from the transect for all mounds, regardless of the number present. To record the distances for the additional mounds (beyond 50), start a second datasheet and number the mounds consecutively, starting with number 51

7. Comments to include in the "notes" section of the datasheet include the presence of inactive mounds, the location of a mound at the base of (or near) a milkweed plant, observations of fire ant predation on monarchs (indicate monarch life stage involved: egg, 1st instar, 2nd instar, 3rd instar, 4th instar, 5th instar, pupa, adult), and other observations of potential importance as determined by the observer(s).

Modification for Linear Sites (Rights-of-Way)

The starting point for the belt transect will need to be located 5m from the corner/edge of the plot so that the road itself is not included in the belt transect. The same applies to the fence line, or other boundary.

Tips for Identifying RIFA Mounds

 $(modified\ from\ \underline{http://articles.extension.org/pages/11278/identifying-fire-ants}\ and \underline{http://articles.extension.org/pages/11055/fire-ant-morphology-reproduction-and-development)}$

- Mounds consist of fluffy, worked soil, particularly a few days after a heavy rain (Figure SOP-6.1). Mounds have no opening in the center, which is a difference from the mounds of most other ant species.
- Imported fire ants leave and enter the mound through underground tunnels, radiating from the mound. In hard compact soils, these tunnels may be visible on the surface. Fire ant foraging trails may also be present and extend many feet from the mound (Figure SOP-6.2).
- Most mounds in turfgrass are just a few inches tall, but undisturbed mounds can reach 18-24 inches in height. The aboveground portion of the mound is just a small part of the fire ant nest, and the below ground portion of the colony can extend several feet deep. Soil type can affect mound size. Mounds in clay soil are often larger than mounds in sandy soil.
- Mounds are often built up against structures or may be located at sites with less frequent disturbance (e.g., fence rows). Mounds may form at the base of *Asclepias* plants (Figure SOP-6.3), as well as other plants.
- Fire ants can move their nests/mounds both horizontally and vertically to optimize access to favorable temperature and humidity. During extreme heat and drought, the ants move deeper into the ground; mounds may lose their domed appearance and be less noticeable.
- RIFA vary in size within a colony, with workers ranging from about 1/8 to 1/4 inch long (Figure SOP-6.4A). RIFA can also vary in color from red to brown (Figure SOP-6.4B).
- Fire ant brood are located near the top of the mound and may be visible after disturbing a mound (Figure SOP-6.5).
- Mounds remain visible after fire ants have been eliminated (Figure SOP-6.6), so it is important to evaluate if ants are present to identify active mounds.

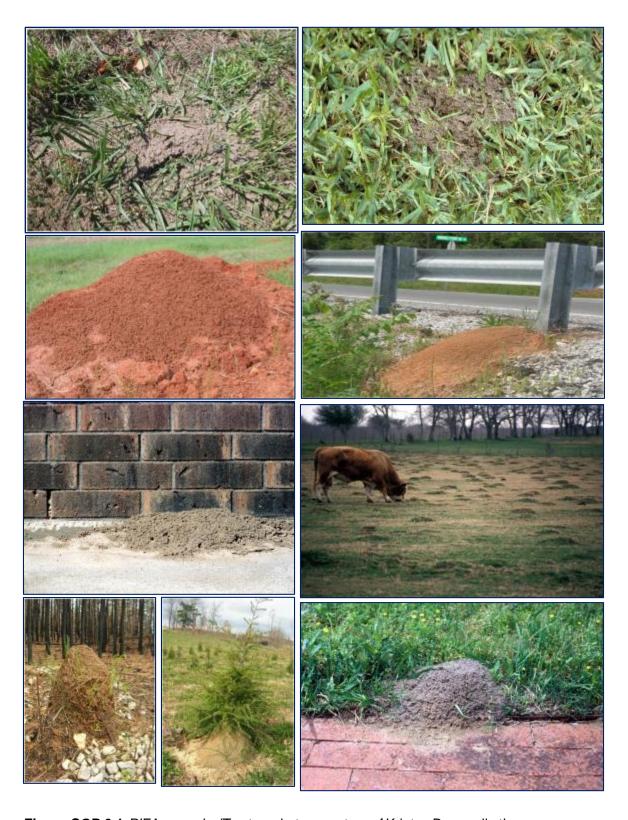


Figure SOP-6.1. RIFA mounds. (Top two photos courtesy of Kristen Baum; all other photos from http://bugmugs.org/)





Figure SOP-6.2. RIFA foraging trails. (Photos from http://bugmugs.org/)





Figure SOP-6.3. RIFA mounds at the base of *Asclepias asperula* (A) and *A. viridis* plants (B). (Photos courtesy of Lee Davis).

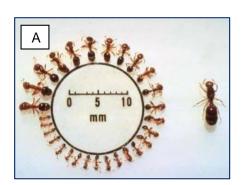






Figure SOP-6.4. Size variation within a RIFA colony (A), color variation (B), and a close-up view of a RIFA (C). (Photos from http://bugmugs.org/)



Figure SOP-6.5. RIFA brood (white objects). (Photo courtesy of Kristen Baum)



Figure SOP-6.6. Mounds remain visible after RIFA have been eliminated (right side of image) unless leveled (left side of image). (Photo from http://bugmugs.org/)